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Editorial Comments

Complement inhibition in renal diseases

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Introduction

The activation of complement and its deleterious consequences have been observed in many renal diseases. Complement contributes to injury in several forms of glomerulonephritis (GN), in acute and chronic humoral rejection after renal transplantation, and at the time of ischaemia/reperfusion injury. It is also thought to accelerate the progression of chronic renal damage. Most of the evidence for the role of complement in enhancing injury comes from experimental data obtained in murine models, in which the addition of complement inhibitors blocks or reduces damage. The first evidence that excessive complement activation can be reversed in humans has been recently reported by Remuzzi *et al*. [1]. These authors reported a combined liver and kidney transplantation in a young child with haemolytic uraemic syndrome (HUS) and a mutation of factor H by which they were able to restore the defective factor H activity with no recurrence of the disease. The biological markers of complement activation normalized after transplantation as well.

In contrast to inhibitors of the coagulation cascade, the clinical application of complement inhibitors has not yet started; this might explain why so little attention has been given by clinicians to this field. With major progresses made in the last 10 years on the engineering of molecules, it is likely that complement inhibition will be introduced in clinical practice soon. Here we would like to emphasize some of the aspects of complement relevant to renal diseases, for which specific inhibitors of complement might produce beneficial effects, but also the possible caveats related to the inhibition of an enzymatic cascade central to the normal function of the innate immune system.

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Complement

The complement system consists of more than 20 proteins, which interact in a cascade sequence (Figure 1). The biological effects of this system include: (i) opsonization and phagocytosis mainly due to the covalent binding of C3b to the targets; (ii) inflammation through the production of anaphylaxis (C3a, C5a) and chemotaxis (C5a), with C5a playing the major role; (iii) insertion of the membrane attack complex (MAC = C5b–9 complex) on pathogens, but also on bystander cells, which are activated; (iv) enhancement of the immune response by the covalent binding of C3 fragments [2]; (v) removal of immune complexes (ICs) by blocking their formation and dissolving large immune aggregates, or by delivering ICs to the fixed macrophages [3]; and (vi) silent removal of apoptotic bodies by C1q and classical pathway activation [4,5].

The system may be activated via the classical, alternative or lectin pathways. The classical pathway is activated by the binding of complement component C1 to the Fc portions of antibody molecules complexed

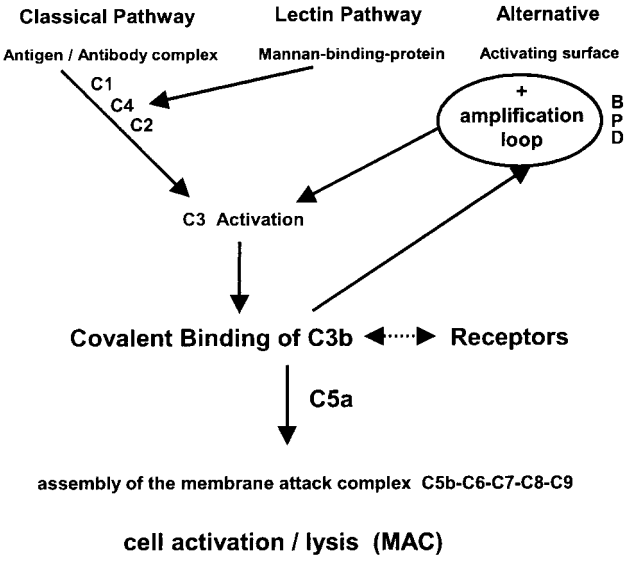

Fig. 1. Complement activation.

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with antigen. The lectin pathway is activated by the binding of mannan-binding lectin (which is structurally related to C1q) to bacterial carbohydrates. The resulting activation of C3 can be amplified by a positive feedback loop, which is also called the alternative pathway since its continuous low-grade physiological activation can be amplified directly on a pathogen or a foreign surface where no complement regulatory proteins are present.

Complement regulators

Complement activation is tightly regulated by a number of circulating and cell-bound proteins. This regulation is delicately balanced between the need to effectively destroy invading microorganisms and to prevent excessive activation of autologous cells and tissues. Three of these regulators inhibit complement at its most crucial step, i.e. the formation of the C3/C5 convertases; thus, they have the potential to abrogate most biological functions of complement including the release of C5a and formation of MAC. Whereas decay accelerating factor (DAF, CD55) is expressed only at low levels in glomeruli and tubules, membrane-cofactor protein (MCP, CD46), is present on almost all cells in the kidney. Interestingly, complement receptor 1 (CR1, CD35), which is on a molar basis the most powerful inhibitor of the C3 convertases, is expressed only on podocytes. The fourth regulator, CD59, interferes with the insertion of the C5b–9 complex in the cell membrane and is present on glomerular and distal tubular cells, but less so on proximal tubules. In addition to these membrane-bound inhibitors, many other soluble proteins in plasma and extravascular fluids may down-regulate complement, e.g. factor H, which blocks activation at the C3 convertase step, and clusterin which interferes with the normal assembly of MAC.

Complement in glomerulonephritis

Although it has been known since the advent of immunofluorescence techniques that many types of GN are characterized by deposition of complement proteins such as C1q, C4 and C3 fragments and MAC, their role in initiating or mediating local damage has remained controversial in human diseases. Of interest for the present discussion are reports analyzing some rare conditions of continuous activation of the alternative pathway that result in glomerular damage. The deficiency of factor H is responsible for a loss of control of the alternative pathway with continuous activation of C3. In pigs, factor H deficiency leads to the development of membranoproliferative GN (MPGN) with dense deposits, i.e. a syndrome very similar to the human MPGN type II observed in patients with nephritic factor (NeF) and in some very rare patients with factor H deficiency as well [6]. The administration to the pigs of purified factor H, so as to keep the alternative pathway under control, prevents the formation of the immune deposits and subsequent glomerular damage, and even when administered later, allows a regression of the nephritis [7]. Patients with NeF, an IgG autoantibody that prolongs the enzymatic half-life of the C3 convertase (C3bBb) thus producing continuous C3 activation in plasma, develop MPGN in $\sim 50\%$ of cases. In this condition, the main glomerular deposits consist of C3 fragments as well, and the clinical syndrome is one of heavy proteinuria and progressive loss of renal function resulting in end-stage renal disease. Thus, complement blockade is a real target for therapy in these exceptional diseases, in which little immunoglobulin (Ig) deposits are found. In contrast, most human GN are characterized by Ig deposits (IgA, membranous, membranoproliferative and lupus GN), so that complement activation is very probably secondary to the local deposition or formation of immune aggregates. Although various models in rabbits and mice indicate that under many circumstances complement is directly involved in local injury, it may be that in some cases local complement activation may not be deleterious. Recent data obtained in C3 knockout mice suggest that complement may have protective functions by preventing local accumulation of immune complexes [8], particularly in the autologous phase of nephrotoxic nephritis induced by heterologous anti-GBM antibodies. Robson *et al.* [9], using C1q knockout mice, provided evidence that C1q plays a direct role in preventing inflammation and damage in a similar model. Such a protective role for complement activation, i.e. dissolution of immune aggregates, was suggested many years ago by Bartolotti *et al.* [10] and later by Furness and Turner [11]. In conclusion, although complement-blocking agents might be beneficial in various forms of GN, inhibition of complement should be performed only under strict clinical investigation protocols.

Ischaemia/reperfusion injury

In most organs, including the heart and the intestine, the ischaemia/reperfusion injury is best explained by the presence of natural Ab, which recognize novel epitopes expressed by endothelial cells and activate complement by the classical pathway [12]. The situation might be different in the kidney, where neither natural Ab [13] nor the classical pathway of complement [14] appears to be involved. Tubular cells are more sensitive to hypoxia than endothelial cells so that the damage is mainly localized to tubules rather than vessels [14]. Tubular cells when hypoxic/necrotic were shown to activate complement by the alternative pathway, and most of the local injury was due to the assembly of the membrane attack complex. It is important to realize that tubular cells produce all

necessary proteins for alternative pathway function, which may explain why ischaemia/reperfusion injury in the kidney is very resistant to complement inhibitors [15]. Furthermore, many other mediators may participate in tubular necrosis in ischaemia/reperfusion injury. The data do not exclude a role for complement in ischaemia/reperfusion injury in humans, which might be different from the animal models studied, but the evidence to date does not favour such a hypothesis. Even in the immediate post-transplantation period, the occurrence of acute tubular necrosis, which is most frequently due to ischaemic injury, is not accompanied by evidence of complement activation on endothelial cells in the kidney; in particular, there is no C4d deposition in peritubular capillaries [16].

Antibody- and complement-mediated rejection after renal transplantation (humoral rejection)

In recent years, the role of alloAb-mediated acute and chronic rejection has been emphasized with evidence for complement activation by the classical pathway being provided mainly by the peritubular capillary deposition of C4d, the fragment of C4 that binds covalently to nearby structures [17]. However, one very rarely finds other complement fragments at the same localization, which is somewhat surprising, since C3 has the same capacity as C4 to bind covalently to local structures. The experimental models used to date have shown clearly that Ab-mediated rejection is complement dependent, although in these models it is not difficult to demonstrate C3 deposition in the kidney. Thus, while there is no doubt that C4d is a good marker for Ab-mediated humoral rejection, what remains undetermined is whether the activation and deposition of C4 is followed by damaging complement activation or not. Only further clinico-pathological investigations will provide us with an answer. In clinical renal transplantation, ~5–10% of recipients suffer from acute humoral rejection, and the rejection episode is often resistant to steroid and antilymphocyte therapy [18]. New therapeutic approaches using plasmapheresis, tacrolimus, mycophenolate mofetil and intravenous immunoglobulin (IVIg) have been shown to be useful in treating acute humoral rejection, but complement inhibitors have not been used in this setting in humans [19]. Another interesting aspect will be that complement inhibitors may block hyperacute rejection due to natural Ab, such as blood group Ab. This situation is identical to the hyperacute rejection seen during the first minutes of xenotransplantation, where the immediate deposition of natural Ab (mainly IgM in humans) brings about an explosive and devastating activation of complement with ensuing endothelial damage, followed by bleeding and thrombosis. In contrast to ischaemia/reperfusion injury in the kidney, where the alternative pathway is mainly damaging, it is evident that to treat or prevent antibody/complement-mediated damage after renal transplantation the classical pathway has to be inhibited, and this at a very early phase of the enzymatic cascade [20,21].

Progression of renal damage

Complement may contribute to the progression of tubulointerstitial injury in renal diseases with proteinuria, since the filtered complement proteins are activated at the surface of proximal tubular cells by the alternative pathway [22]. Complement participates in the renal damage in various models of chronic diseases such as hypertensive nephropathy [23]. Clusterin knockout mice develop a chronic progressive glomerulopathy characterized by mesangial immune deposits, suggesting that under physiological conditions, clusterin protects against glomerular damage [24]. Increasing the local expression of clusterin has shown beneficial effects in ischaemic brain damage [25,26], and interestingly the expression of clusterin is up-regulated in nephritis [27]. Taken together, the data are suggestive that complement blockade might be useful in the prevention of chronic damage. Because this is an important clinical problem, even small improvements in the general strategies to fight chronic progression of renal damage might be important for patients.

Complement inhibition

A complete and long-term inhibition of complement may have some detrimental side effects, as suggested by observations made in patients with various complement deficiencies. Such patients have an increased susceptibility to infection (mainly encapsulated bacteria including meningococci) and immune-complex diseases. However, in many human diseases only short-term inhibition would be needed, e.g. in ischaemia/reperfusion injury or in acute humoral rejection in transplantation. In addition, it is quite possible that down-regulation rather than blockade of complement may be sufficient on many occasions. Thus, although we should be cautious with inhibiting complement, it would be unwise not to start clinical investigations because of potential side effects which may be prevented (e.g. immunization against meningococci) or for which a close surveillance can be proposed. For the time being some complement inhibitors have already reached clinical phase I and II studies. Two of them are soluble recombinant CR1 and single-chain Fv antibody against C5 [28].

It has not been the purpose here to discuss further complement inhibitors [29], but rather to emphasize the clinical conditions in which complement is directly responsible for renal damage. Clinical studies require large financial investments nowadays, and few pharmaceutical companies are ready to engage on large studies

without any initial evidence for a possible beneficial effect. It is unlikely that nephrologists will be the first to handle complement inhibitors even in clinical research areas, since the possible positive effects of complement blockade in the many diseases described here may not be readily apparent, either because it would require the inclusion of many patients (blockade of C5b–9 in ischaemia/reperfusion, humoral rejection) or because long-term studies would be required (GN or progression of renal disease). There is, however, some hope that once more rare diseases might bring the proof of principle required. In patients with factor H deficiency, soluble recombinant CR1 may block immediately the excessive activation of complement and restore C3 levels. In patients with GN, long-term inhibition of complement could prevent further deterioration of renal function, and in some patients with HUS, might block the clinical syndrome.

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